Electrochemical sensing of etoposide by using carbon quantum dots modified glassy carbon electrode

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1. Experimental

1.1 Particle size and zeta-potential analysis

Particle size and zeta-potential of CQDs were measured by Zetasizer MALVERN (Malvern Instruments Ltd. Worcestershire WR14 1XZ, United Kingdom). The particle size measurements were performed considering a refraction index of the dispersive phase of 2.418 and 1.333 for the dispersive environment. The absorption coefficient in both cases was 10^{-3}. The measuring temperature was set at a constant value of 25 °C, while the viscosity was 0.8872 g m^{-1} s^{-1}. For each measurement, disposable cuvettes type ZEN 0040, were used, containing 40 µL of a sample. The equilibration time was 120 s, at a measurement angle of 173° backscatter. The measurements were performed in triplicate. The zeta-potential analysis was performed considering the same refraction index and absorption coefficient as described in particle size measurements above. Furthermore, the measuring parameters such as, temperature and viscosity were the same as in particle size measurements. Calculations considered the diminishing of particles concentration based Smoluchowsky model, with a F (κa) of 1.50 and an equilibrating time of 120 s. For the measurements, a disposable cell DTS1070 was employed. In each case, the measurement duration depended on the number of runs, which varied between 20 and 40. The measurements were carried out in triplicate and were performed under the automatic setting of attenuation and voltage selection.

1.2 Fluorescence and UV/VIS spectroscopy

Fluorescence and absorbance spectra were acquired by a multifunctional microplate reader Tecan Infinite 200 PRO (TECAN, Zurich, Switzerland). Excitation wavelength for CQDs was 280 nm. The fluorescence scan was measured within the range from 250 to 650 nm per 2-nm steps. The detector gain was set to 100. Absorbance was measured within the range from 250 to 400 nm. The samples for both measurements (50 µL) were placed in a transparent 96 well microplate with flat bottom (Thermo Scientific, Waltham, MA, USA). All measurements were performed at 25 °C controlled by the Tecan Infinite 200 PRO.

1.3 Flow injection analysis with electrochemical detection

Flow injection analysis with an electrochemical detection (FIA-ED) system consisted of two chromatographic pumps (Model 582, ESA) with working range 0.001 – 9.999 mL min^{-1} and the CoulArray electrochemical detector (Model 5600A, ESA). The detector consisted of flow analytical chamber (Model 6210, ESA). The chamber contains four analytical cells. One analytical cell contains two references (hydrogen-palladium), two counter electrodes and one porous graphite working electrode. The electrochemical detector is situated in control module, which is thermostated. Sample (20 µL) was injected by manual valve (Rheodyne, Oak Harbor, WA, USA). Flow rate of mobile phase was 1 mL min^{-1}.

1.4 High performance liquid chromatography with electrochemical detection

Chromatographic analysis was performed using high performance liquid chromatography with electrochemical detection (HPLC-ED). The HPLC-ED system consisted of two solvent delivery pumps operating in the range of 0.001 – 9.999 mL min^{-1} (Model 582, ESA), and a chromatographic column Kinetex 5µm EVO C18 (150 × 46 mm,

Phenomenex Inc., CA, USA) and a CoulArray electrochemical detector (Model 5600A, ESA). The electrochemical detector includes one flow cell (Model 6210, ESA). The cell consists of four working carbon porous electrodes, each one with auxiliary and dry Pd/H_2 reference electrodes. Both the detector and the reaction coil/column were thermostated. The sample (20 µL) was injected using autosampler (Model 542 HPLC, ESA). Samples were kept in the carousel at 8 °C during the analysis. The column was thermostated at 35 °C. The mobile phase consisted of 80 mM trifluoroacetic acid (TFA) and 100% acetonitrile (*v*/*v*, ACN). The compounds of interest were separated by the following linear gradient: 0 → 7 min (3% ACN), 7 → 15 min (15% ACN), 15 → 25 min (30% ACN), 25 → 33 min (98% ACN). The mobile phase had a flow rate of 1 mL min^{-1} and the working electrode potential was set to 900 mV.

1.5 Scanning electron microscopy (SEM) analysis

The morphology of the bare GCE, GCE/CQDs, and GCE/CQDs/ETO were examined using scanning electron microscope MIRA3 LMU (Tescan, a.s., Brno, Czech Republic). This model is equipped with a high brightness Schottky field emitter for low noise imaging at fast scanning rates. The SEM was fitted with Everhart-Thronley type of the SE detector, the high speed YAG scintillator-based BSE detector. An accelerating voltage of 15 kV and beam current about 1 nA were used with satisfactory results regarding to its maximum throughput.

1.6 Fluorescence microscopy (FM) analysis

Microscopic studies of the bare GCE, GCE/CQDs, and GCE/CQDs/ETO were performed using an inverted Olympus IX 71S8F-3 fluorescence microscope (Olympus, Tokyo, Japan) equipped with a mercury arc lamp X-cite 120 Lamp (120 W, Lumen Dynamics, Mississauga, Canada) for illumination. The images were taken using a Camera Olympus DP73 (Olympus, Tokyo, Japan) and processed by Stream Basic 1.7 software (Olympus Soft Imaging Solutions GmbH, Münster, Germany) using a resolution of 1 600 × 1 200 pixels. A 4X-objective lens was used to cover most of the part of the electrode in a single frame.

2. Results and discussion

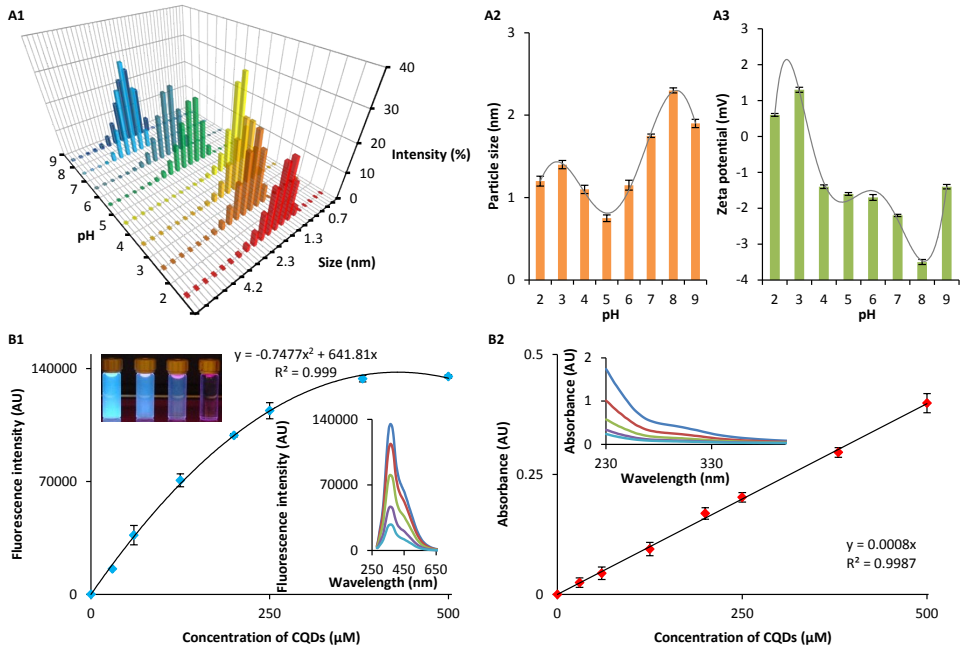

Fig. 1S: Characterization of CQDs (200 µM) using Zetasizer and Fluorimeter. (A1) Size distribution by intensity of CQDs in different pHs of phosphate buffer. Dependence of (A2) particle size and (A3) zeta potential of CQDs on pH of phosphate buffer. Dependence of (B1) fluorescence intensity and (B2) absorbance on concentration of CQDs. Fluorescence (inset in B1**)** and absorbance (inset in B2**)** spectra of CQDs.

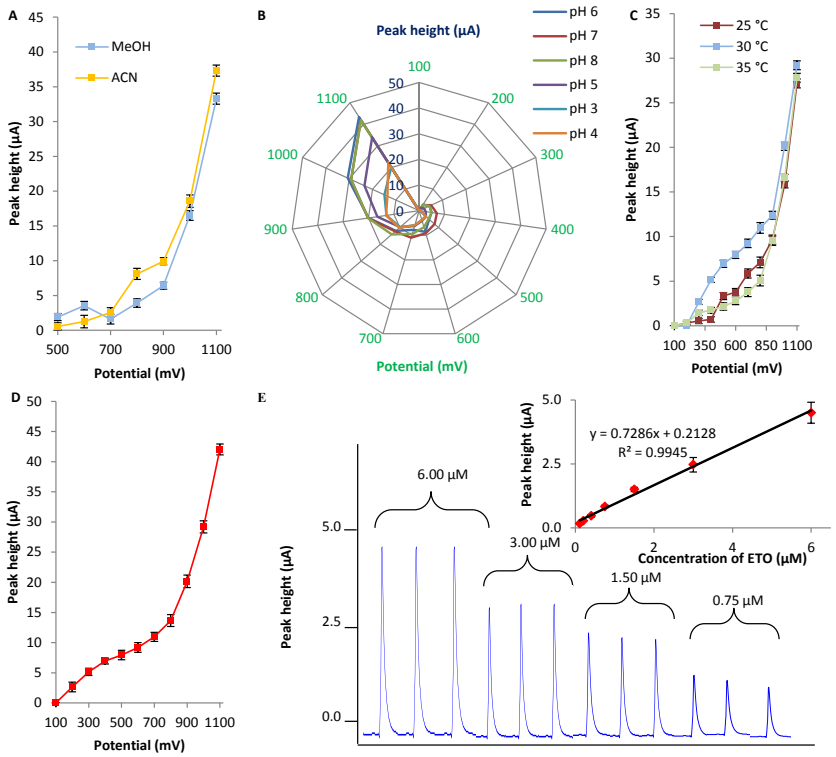

Fig. 2S: Optimization of FIA-ED of ETO. Applied concentration of ETO for analyses instead of calibration curves was 3 µM. (A) Influence of addition of organic solvents (MeOH and ACN) on amperometric signal of the ETO. (B) Optimization of potential for detection (100 – 1100 mV) of ETO, and its behaviour under different pH values of BR buffer. (C) Optimization of temperature influence on detection of ETO. (D) Hydrodynamic voltammogram of ETO measured under the optimized conditions. (E) Array record of ETO calibration curve measured within the range from 0.75 to 6.00 µM. Calibration curve of ETO measured under the optimized condition (inset in E).